

**CLAIMS**

1. A method for screening agents modulating I $\kappa$ B $\alpha$  protein ubiquitination by a functional ubiquitin ligase protein complex containing  $\beta$ -TrCP protein, said method comprising the following steps:
  - (a) bringing into contact a candidate agent to be tested with recombinant yeast 5 cells that express in their nucleus:
    - (i) a fusion protein containing the polypeptide I $\kappa$ B $\alpha$  and at least one first detectable protein; and
    - (ii) a protein containing the polypeptide  $\beta$ -TrCP;
  - (b) quantifying said first detectable protein in the yeast cells, at the end of at 10 least one predetermined period of time after bringing the candidate agent into contact with said cells;
  - (c) comparing the result obtained in step (b) with a control result obtained when step (a) is carried out in the absence of the candidate agent.
- 15 2. A method according to claim 1, characterised in that step (a) includes the following steps:
  - (a1) growing yeast cells which express in their nucleus a fusion protein containing the polypeptide I $\kappa$ B $\alpha$  and at least one first detectable protein;
  - (a2) stopping the expression of said fusion protein containing the polypeptide 20 I $\kappa$ B $\alpha$  and at least one first protein detectable by the yeast cells;
  - (a3) bringing the yeast cells obtained at the end of step (a2) into contact with the candidate agent to be tested.
- 25 3. A method according to claim 2, characterised in that the yeast cells express the protein containing the polypeptide  $\beta$ -TrCP throughout all the steps (a1), (a2) and (a3).

4. A method according to claim 2, characterised in that the yeast cells express the protein containing the polypeptide  $\beta$ -TrCP throughout all the steps (a2) and (a3) and do not express the protein containing the polypeptide  $\beta$ -TrCP in step (a1).  
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5. A method according to claim 2, characterised in that the yeast cells express the protein containing the polypeptide  $\beta$ -TrCP throughout all the steps (a2) and (a3), and
  - 10 (i) do not express the protein containing the polypeptide  $\beta$ -TrCP for a predetermined time at the start of step (a1);
  - (ii) do express the protein containing the polypeptide  $\beta$ -TrCP for the remainder of step (a1).
6. A method according to any one of claims 1 to 5, characterised in that the  
15 detectable protein in the polypeptide containing  $I\kappa B\alpha$  polypeptide is chosen from an antigen, a fluorescent protein and a protein having enzymatic activity.
7. A method according to claim 6 characterised in that the detectable protein is a  
20 fluorescent protein selected from the GFP protein or one of its derivatives, the YFP protein or one of its derivatives, and the dsRED protein.
8. A method according to claim 6, characterised in that the detectable protein is a protein having enzymatic activity selected from luciferase and  $\beta$ -lactamase.
- 25 9. A method according to claim 6, characterised in that the detectable protein is an antigen selected from the Ha peptide and the Flag peptide.
10. A method according to any one of claims 1 to 9, characterised in that the  
30 protein containing the polypeptide  $\beta$ -TrCP is a fusion protein also containing a second detectable protein.

11. A method according to claim 10, characterised in that the second detectable protein included in the fusion protein containing the  $\beta$ -TrCP polypeptide is chosen from an antigen, a fluorescent protein and a protein having enzymatic activity.
12. A method according to any of claims 10 and 11, characterised in that (i) the first detectable protein included in the fusion protein containing the polypeptide I $\kappa$ B $\alpha$  and (ii) the second detectable protein included in the fusion protein containing the polypeptide  $\beta$ -TrCP are different from each other.
13. A method according to any one of claims 1 to 12, characterised in that the protein containing the polypeptide I $\kappa$ B $\alpha$  also contains a nuclear localisation peptide.
14. A method according to any one of claims 1 to 13, characterised in that the protein containing the polypeptide  $\beta$ -TrCP also contains a nuclear localisation peptide.
15. A method according to any one of claims 1 to 14, characterised in that the protein containing the polypeptide I $\kappa$ B $\alpha$  is the protein with the sequence SEQ ID N°2.
16. A method according to any one of claims 1 to 15, characterised in that the protein containing the polypeptide  $\beta$ -TrCP is the protein with the sequence SEQ ID 4.
17. A method according to any one of claims 1 to 16, characterised in that at step (b), when the first detectable protein is an antigen, said first detectable protein is

quantified by detecting the complexes formed between said protein and the antibodies which recognise it.

18. A method according to any one of claims 1 to 16, characterised in that at step  
5 (b), when the first detectable protein is a fluorescent protein, said detectable protein is quantified by measuring the fluorescent signal emitted by said protein.

19. A method according to any one of claims 1 to 16, characterised in that at step  
10 (b), when the first detectable protein is a protein having enzymatic activity, said detectable protein is quantified by measuring the quantity of substrate modified by said protein.

20. A method according to any one of claims 1 to 19, characterised in that the recombinant yeast cells are transformed with:

15 respectively:

- (1) a first polynucleotide that contains (a) an open reading frame coding for (i) the fusion protein containing, the I $\kappa$ B $\alpha$  polypeptide and (iii) a first detectable protein, and a regulatory sequence functional in yeast cells which controls expression of said open reading frame; and
- 20 (2) a second polynucleotide that contains (a) an open reading frame coding for (i) the protein containing the  $\beta$ -TrCP polypeptide, ii) a nuclear localisation sequence and (iii) a regulatory sequence functional in yeast cells which controls expression of said open reading frame;

25 21. A method according to claim 20, characterised in that the regulatory sequence contained in the first polynucleotide, the regulatory sequence contained in the second polynucleotide, or both regulatory sequences, contain a promoter functional in yeast cells and sensitive to the action of an inducing agent.

22. A method according to claim 21 characterised in that the inducible promoter functional in yeast cells is chosen from *PGK1*, *ADH1*, *TDH3*, *LEU2* and *TEF1*.
23. A method according to claim 21 characterised in that the inducible promoter functional in yeast cells is chosen from *CUP1*, *GAL1*, *MET3*, *MET25*, *MET28*, *SAM4* and *PHO5*.
24. A method according to any one of claims 20 to 23, characterised in that the first polynucleotide contains the regulatory sequence *GAL1*, which activates the expression of the open reading frame coding for the fusion protein containing the polypeptide the I $\kappa$ B $\alpha$  polypeptide in the presence of glucose.
25. A method according to the claim 20 to 23, characterised in that the second polynucleotide contains the regulatory sequence *CUP1*, which activates the expression of the open reading frame coding for a protein containing the polypeptide  $\beta$ -TrCP in the presence of copper sulphate.
26. A method according to any one of claims 20 to 25, characterised in that the recombinant yeast cells have the first and second polynucleotide in a form integrated into their genome.
27. A method according to any one of claims 1 to 26, characterised in that the recombinant yeast cells have in their genome an inactivated form of one or several genes which controls the expression of transporter proteins in the plasma membrane.
28. A method according to claim 27, characterised in that the inactivated genes are chosen from genes *PDR1* and *PDR3*.
29. An expression cassette functional in yeast cells containing a coding polynucleotide which includes an open reading frame encoding the fusion

protein which contains the polypeptide the I $\kappa$ B $\alpha$  polypeptide and at least one first detectable protein, and a regulatory sequence functional in yeast cells which controls the expression of said open reading frame.

- 5        30. An expression cassette functional in yeast cells including a polynucleotide which contains an open reading frame encoding a protein containing the  $\beta$ -TrCP polypeptide and a regulatory sequence functional in yeast cells which controls the expression of said open reading frame.
- 10      31. An expression cassette according to one or other of claims 29 and 30, characterised in that the regulatory sequence contained in said polynucleotide, the regulatory sequence contained in the second polynucleotide, or both regulatory sequences, contain a promoter functional in yeast cells and sensitive to the action of an inducing agent.
- 15      32. An expression cassette according to claim 31 characterised in that the inducible promoter functional in yeast cells is chosen from *PGK1*, *TEF1*, *PHO5*, *MET3*, *MET28*, *CUP1*, *GAL1* and *SAM4*.
- 20      33. An expression vector characterised in that it contains an expression cassette according to any one of claims 29 to 32.
34. An expression vector according to claim 33, characterised in that it is the vector pCSY226-NLS-I $\kappa$ B $\alpha$ .
- 25      35. An expression vector according to claim 33, characterised in that it is the vector pCSY226-NLS- $\beta$ -TrCP.
- 30      36. A recombinant yeast strain containing, in a form integrated into its genome,  
          (i) a first polynucleotide that contains an open reading frame coding for the fusion protein containing the polypeptide the I $\kappa$ B $\alpha$  polypeptide, and at least one

- first detectable protein, and a regulatory sequence functional in yeast cells which controls expression of said open reading frame; and
- (ii) a second polynucleotide that contains an open reading frame coding for a protein containing the  $\beta$ -TrCP polypeptide and a regulatory sequence functional in yeast cells which controls expression of said open reading frame.
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37. A recombinant yeast strain according to claim 36, characterised in that it consists of the yeast strain CYS135 deposited in the Collection Nationale de Cultures of microorganisms at the Institut Pasteur de Paris (CNCM) under 10 accession number I-3187.
38. The tools or kit for screening agents modulating the ubiquitination of the  $I\kappa B\alpha$  protein by a functional ubiquitin ligase protein complex containing the  $\beta$ -TrCP protein, characterised in that it contains
- 15 (i) a first expression vector containing an expression cassette according to claim 29; and
- (ii) a second expression vector containing an expression cassette according to claim 30.
- 20 39. The tools or kit for screening agents modulating the ubiquitination of the  $I\kappa B\alpha$  protein by a functional ubiquitin ligase protein complex containing the  $\beta$ -TrCP protein, characterised in that it includes recombinant yeast cells containing, in a form integrated into their genome, respectively
- (i) an expression cassette according to claim 29; and
- 25 (ii) an expression cassette according to claim 30.
40. The tools or kit according to claim 39, characterised in that it contains recombinant yeast cells of the strain CYS135 deposited at the CNCM under accession number I-3187.